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The Importance of Branched-chain Amino Acids and Nitrate in Sports Performance and Health

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Abstract Branched-chain amino acids (BCAA) and nitrate have become increasingly popular mainly for their potential effect on individuals' health and secondary as ergogenic aids. The purpose of this narrative review was to incorporate the current scientific evidence of BCAA and nitrate supplementation on athletic performance and health. The current recommendations of BCAA and nitrate supplementation are discussed, as well as possible health complications associated with its intake. Pubmed, Scopus, and Web of Science were searched for articles on the effects of BCAA and nitrate supplementation in humans. The positive effect of BCAAs supplementation on athletic performance does not appear to be fully established. BCAAs supplementation seems to be recommended for all athletes who exercise vigorously daily, as it enhances their recovery after causing exercise-induced muscle damage. BCAAs supplementation reduces the feeling of delayed muscle pain, which follows because of muscle damage. Limited scientific data suggest a potentially beneficial effect of BCAAs on reducing central fatigue. Several clinical conditions could benefit from BCAAs' consumption. However, there are no reliable markers to evaluate or quantify their requirements. There are no exact consumption protocols and absolute recommendations, mainly because BCAAs are also obtained through the consumption of animal protein. Dietary nitrates lower blood pressure, reduce the cost of exercise oxygen, and, at least sometimes, enhance exercise capacity. Taking supplements for 2-6 days (or up to 15 days) can increase athletic performance during high-intensity exercise. The duration of continuous maximum exercise for which nitrates appear to be ergogenic is between 5-30 min. There is limited evidence that nitrates are beneficial in prolonged exercise performance (40 min), at least when administered short term. Supplementation of approximately 5-9 mmol of nitrate/day for 1-15 days may have beneficial effects on normal exercise responses, although the exact dose-response relationship has not yet been established. Five to 9 mmol of nitrates can be easily consumed in the normal diet and there is currently no evidence to adequately document that taking additional nitrates produces greater benefits. The effectiveness of acute nitrate supplementation is likely to depend on many factors, such as sex, health, hypoxia, diet, and level of fitness/training experience of the subjects. Nitrate needs are most likely met by ingesting approximately 250-500 g of leafy and root vegetables per day; however, dietary supplements might represent a more convenient and accurate way of covering an athlete's nitrate needs.

Keywords: *branched-chain amino acids, nitrates, exercise performance, health, recommendations*

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1. Introduction

In the last decade, the study of the ergogenic effects of dietary supplements has been a major topic in athletic performance. Athletes mainly consume sports foods and nutritional supplements because it will offer them benefits, such as (a) the promotion of exercise training adjustments and competitive performance; (b) the augment in energy reserves; (c) the promotion of recovery among sessions; (d) the protection of health issues (e.g. illness, chronic fatigue, and injury); and (e) the consumption of an effective source of nutrients. [1] Two of the most effective and safest dietary supplements in enhancing health and athletic performance are branched-chain amino acids (BCAA) and nitrate. Increased requirements for intake of high-quality protein (the source of amino acids) can improve athletic performance. [2] Among amino acids that are of special significance for exercising individuals include BCAA. [2] BCAA comprises essential amino acids leucine, isoleucine, and valine. BCAA account for about one-third of muscle proteins and they are essential because they must be obtained from the diet as they cannot be synthesized de novo. [3] It is probable that because the metabolism of BCAA takes place within the

muscles, ergogenic properties may be derived. [3] Specifically, among the three BCAAs, leucine, in particular, appears to play an important role in activating protein synthesis and insulin secretion, improving athletic performance, decreasing central fatigue, and deteriorating exercise-induced muscle injury. [4] BCAA intake reduces tryptophan uptake by the central nervous system, decreases protein loss, and maintains post-exercise glutamine concentration that would be involved in the lessening of immune suppression observed after the exercise training. [5,6,7] BCAAs help affect health as it is considered that spare lean body mass during weight loss, enhance wound healing, promote muscle protein anabolism with aging, and have beneficial effects in the setting of renal and liver failure because they are predominantly metabolized in muscle than the liver. [8,9,10,11] Also, BCAA is an essential source of nitrogen for the synthesis of non-essential amino acids. [2]

One of the most popular ergogenic supplements among athletes is nitrate ions (NO_3^-) or nitrates as they are widely known. The active ingredient in nitrate-containing formulations is nitric oxide (NO). Nitric oxide (NO) is a signaling molecule involved in several cellular and vascular and cellular functions, such as vasodilation, angiogenesis, and cellular respiration. [12] It is produced through both endogenous and exogenous pathways by dietary nitrate (NO_3^-) intake. [13] NO_3^- is considered to have a positive effect on certain indicators of athletic performance, mainly through its action as a vasodilator, as well as health-promoting effects. [14] Nitrate supplements have been the focus of scientific studies mainly concerning their possible positive effect on various health parameters (e.g. blood pressure, cardiovascular events) but also on athletic performance. [15] Nitrate and nitrite were previously considered as primarily final elimination products of NO, however, recent knowledge indicates that they can be converted to NO in vivo. [16] The dietary administration of beetroot juice (a naturally rich source of nitrate) is of extensive interest to athletes and recreational exercisers, while, the International Olympic Committee classified beetroot juice among the five supplements that have a strong evidence base for promoting intense exercise performance. [17] Specifically, current scientific data proposed that NO_3^- can enhance endurance exercise, intermittent high-intensity exercise, and some forms of resistance exercise. [18] Furthermore, dietary NO_3^- has long been recognized for advantageous effects on health such as effects on mitochondrial respiration, improved contractile function, vasculoprotective effects, and effects on fatigue development. [19] Together, the debate over the consumption of legally ergogenic aids such as BCAAs and nitrate has centered on their common role as the above-mentioned substances are thought to positively improve athletic performance in aerobic and/or anaerobic exercise and many aspects of health. [20]

Thus, the present narrative review aims to perform a summary of the most recent scientific literature that has explored the effect of BCAAs and nitrate supplementation in athletic performance, to investigate their potential contribution to health, and to present current recommendations for safe administration.

2. Methods

Studies for this narrative review were found primarily through a systematic search of the electronic databases MEDLINE, PubMed, and EMBASE, using terms, title words, and abstract words such as "exercise training", "athletes", "athletic performance", "nutrition supplements", "ergogenic aids", "branched-chain amino acids" and "nitrate". We also employ past review articles, as well as references from original studies and related books, in our computer analysis. Athletic performance, exercise, health, branched-chain amino acids, and nitrate were all used as keywords. We looked for studies that used the above terms and were published in the English language between January 1985 and January 2022. Studies were considered if they offered information on bicarbonate sodium or b-alanine consumption in athletes, evaluated their potential efficacy in terms of athletic performance and/or health, or detailed the mechanism of action, administration procedures, safety, and recommendations. Each qualified article's title and abstract were examined, and full-text articles were retrieved in circumstances where inclusion was in doubt.

3. Branched-chain Amino Acids

BCAAs are found in significant amounts in all protein-rich foods, especially eggs, poultry, meat, and dairy products. Plant foods such as oats, soy, and lentils are important sources of leucine. Also, meat, chicken, fish, eggs, cheese, nuts, and lentils contain significant amounts of isoleucine, while fish, meat, and some vegetables contain significant amounts of valine. [3]

3.1. Mechanisms of Action

BCAAs are considered to enhance athletic performance through their involvement in many mechanisms. BCAAs are considered important factors in the activation and production of key muscle proteins, which have the final effect of enhancing protein synthesis, with the main contribution of leucine. [21] Specifically, BCAAs are thought to activate protein kinase-AMP which in turn enhances protein synthesis with the result of increased muscle hypertrophy. [21] It is considered that when BCAAs were injected into people at rest, protein balance was increased either by decreasing the rate of protein breakdown, by increasing the rate of protein synthesis, or by combining both. [22] Leucine intake in combination with carbohydrates in men who exercised with resistance training has been shown to lead to a greater increase in protein synthesis compared to intake of the same amount of carbohydrate without leucine. [23] However, the majority of research regarding leucine intake and protein synthesis has been conducted using animal models. BCAA intake is beneficial during aerobic exercise. When BCAAs are taken during aerobic exercise, the net rate of protein breakdown has been shown to decrease. [24] Equally important is the finding that BCAA administration given before and during strenuous aerobic exercise to individuals with low muscle glycogen stores

may also delay muscle glycogen depletion. [24] When BCAAs were given to runners during a marathon they improved the performance of the "slowest" runners (those who completed the race in 3.05 h-3.30 h) compared to the "fastest" runners (those who completed the race in times less than 3.05 h). [25] There are many reported metabolic causes of fatigue, such as glycogen depletion, hydrogen cations accumulation, decrease in phosphocreatine level, hypoglycemia, and an increase in the free tryptophan/BCAA ratio, which can be alleviated by BCAA supplementation. [26,27,28]

During prolonged aerobic exercise, the concentration of free tryptophan increases, and then the tryptophan uptake into the brain increases. When this happens, serotonin is produced, which is thought to play a role in the subjective feeling of fatigue. In particular, serotonin is a neurotransmitter in the human brain, which uses tryptophan as a substrate and causes relaxation, calm and good mood. [29] Similarly, BCAAs are transported within the brain by the same transport system as tryptophan and thus "compete" with the tryptophan that is to be transported to the brain. It is a given that the effects of serotonin are not favorable during exercise as they lead to a reduction in the intensity of the effort and possibly to its permanent cessation. [30] Therefore, it is believed that when certain amino acids such as BCAAs are present in plasma in sufficient quantities, they can theoretically reduce tryptophan uptake into the brain and, ultimately, reduce the feeling of fatigue. [30] In addition to the aforementioned benefits in athletic performance, several researchers concluded that BCAA supplementation can enhance mental performance in athletes after prolonged endurance exercise. [31] Another study has investigated the protective effect of BCAAs on the possible reduction of some intramuscular enzymes thought to be associated with exercise muscle damage. Exercise-induced muscle damage occurs after high-intensity resistance exercise and especially after eccentric muscle contractions. [4] Finally, studies suggest that BCAAs may play an important role in reducing the onset of symptoms of delayed muscle pain. [24] Delayed muscle pain is a result of intense muscle strain, and is considered a determining factor in fatigue of athletic performance, as it makes it difficult to continue the training program of athletes. [12] Conclusively, BCAAs have been shown to help with recovery processes from exercise, such as stimulating protein synthesis, re-synthesizing muscle glycogen, delaying the onset of muscle fatigue, and maintaining mental function in aerobic exercise.

3.2. Effects of BCAA Supplements on Exercise-related Muscle Protein Metabolism

The effects of BCAA supplements before and after exercise on muscle protein metabolism and exercise-induced muscle damage have been studied in humans. [3] In particular, BCAA supplementation (77 mg/kg body weight) before exercise has been reported to increase intracellular and arterial BCAA levels during exercise and lead to suppression of endogenous muscle breakdown. [32] Oral administration of BCAAs (12 g/d for 2 weeks and an additional 20 g before and after each test) has also been reported to suppress the increase in serum creatine kinase activity for several days after exercise. [33] Comparable results were also incorporated in another study in which subjects ingested a mixture of amino acids (containing 3.6 g of amino acids with 37% BCAAs) before and after the exercise test and 2 doses/d of the amino acid mixture for 4 days after the test. [34] The amino acid supplement also reduces the muscle soreness that usually accompanies exercise. [35] Although the mechanism responsible for the protective effects of BCAA supplements against exercise-induced muscle damage and pain has not been fully elucidated, it is thought that stimulation of protein synthesis by leucine and suppression of protein breakdown due to protein breakdown of BCAAs may be significantly involved. [3,28] The most effective ratio of the three BCAAs in terms of their beneficial effects on athletic performance is not sufficiently proven.

3.3. BCAA as Fuel for Exercise

Although early studies have shown that BCAAs can act as fuel during exercise, in addition to carbohydrates and fat, it has been shown that the activities of enzymes involved in the oxidation of BCAAs are too low to allow significant BCAAs' contribution to energy expenditure. [27,36,37] Previous research with a 13C-labeled BCAA concluded that the oxidation of BCAAs increased 2- to 3-fold during exercise, while the oxidation of fats and carbs enlarged 10- to 20-fold. [38,39] Therefore, BCAA supplementation does not significantly contribute as fuel during exercise, and in this respect, its supplementation during exercise is needless. [40]

3.4. BCAA and Protein Recycling Rate

In vitro studies showed that BCAAs supplementation stimulates muscle tissue protein synthesis and inhibits protein degradation. [40] On contrary, in vivo studies in healthy individuals do not support the helpful effect on protein balance observed in vitro. [21,41,42] A review study by Matthews DE (2005) showed a stimulatory effect of BCAAs on muscle protein synthesis and an anabolic effect on the breakdown of muscle protein. [43] Also, leucine, not its metabolites, activates protein synthesis. [44] Scientific data proposed that the simultaneous intake of leucine and protein with carbs stimulates muscle protein synthesis and optimizes protein balance as compared to carbs intake after 45 minutes of resistance training. [45] Therefore, there is ample evidence to suggest that oral ingestion of BCAAs has an anti-catabolic effect during and after exercise, while BCAA supplements could also speed up the recovery of muscle damage after exercise. [36]

3.5. Effect of BCAA on Athletic Performance

BCAA supplementation is widely used among athletes with the assumption of enhanced athletic performance and muscle mass. A previous review study speculated that BCAAs (30-35% leucine) supplementation before or during endurance exercise could prevent or reduce the net rate of protein degradation, may enhance both physical and mental performance, and might present a helpful

effect on glycogen depletion and degradation of muscle glycogen stores. [46] A later review study by Negro et al. (2008) speculated that whether BCAA supplementation could be enhanced athletic performance is not extensively accepted. [47] However, the same review proposed that BCAA is probably a useful supplement for muscle recovery and immune regulation for exercise events. [47] Another review study by Salinas-García et al. (2014) incorporated that BCAA supplementation resulted in minor muscle damage and pain, less mental fatigue and perceived exertion, and improved immune and anabolic response in the recovery period. [48] A most recent review by Plotkin et al. (2021) showed that most of the scientific literature cannot support ergogenic aids of BCAA supplementation in muscle strength and hypertrophy. [49] However, the authors proposed that, based on preliminary results, more research is required on the topic in older individuals. [49] In line with previous results, another recent review by Master et Macedo (2021) concluded that the heterogeneity of the studies and low methodological quality support evidence neither for the BCAA supplementation nor leucine as ergogenic aids for athletic performance. [50] Even if several athletes have a moderately high consumption of BCAA because of their elevated protein and energy intake and/or supplementation, the effect of BCAA on athletic performance is still ambiguous. [40] BCAA is not only mentioned in current guidelines. Conclusively, the ISSN position stand stated BCAAs stimulate protein synthesis, delaying the start of fatigue, support in glycogen resynthesis, and help to maintain mental function in aerobic-based exercise. [51]

3.6. Effect of BCAA on Immune Responses to Exercise

It is believed that prolonged high-intensity exercise training is associated with a temporary immune suppression that affects lymphocytes, macrophages, and neutrophils. [52] The mechanisms involved have not been fully elucidated and shown to be multifactorial, including hormonal actions such as cortisol and catecholamines, changed heat shock proteins expression, inhibition of cytokine production and macrocyte, and decreased concentration of heat shock plasma glutamine. [53] Nitrogen and BCAAs are involved in glutamine synthesis. Studies have evaluated the efficiency of BCAA supplementation in exercise to preserve plasma glutamine levels and alter immune responses. A review study by Negro et al. (2008) speculated that after prolonged high-intensity exercise the supplementation of BCAA recovers blood mononuclear cell proliferation and plasma glutamine concentration, while it modifies the exercise-related cytokine production, resulting in a diversion of the lymphocyte immune response. [47] A study among trained cyclists concluded that as BCAA supplementation blunted the neutrophil response to high-intensity cycling training, potentially, it benefits immune function during a prolonged season. [54] Also, a study revealed that BCAA supplementation before a prolonged intense race prevented the decrease in plasma glutamine concentration as well as modified advantageously the immune response to exercise. [55] Given that other studies have shown that glutamine supplementation in exercise did not prevent the decline of lymphocyte proliferation, these findings need to be considered with carefulness. [56,57]

3.7. BCAA and Health

BCAA shows noteworthy metabolic and regulatory roles. BCAA (especially leucine) promotes protein synthesis through the TOR signaling pathway and is considered as a feed aid to control meat quality (used as ergogenic aids for bodybuilders). BCAA are metabolic regulators in glucose and lipid metabolism. [58] It is considered that promotes mammary health, enhance milk quality and help in early embryo development and implantation. [58] Also, a review study speculated that BCAAs enhance gut health and immunity by up-regulation of pro-inflammatory cytokines and down-regulation of anti-inflammatory cytokines. [58] Moreover, it could act as a biomarker for the early detection of chronic diseases (e.g. diabetes and insulin resistance). Examining the potential role of BCAA in aging, there is disagreement over whether older people require higher amounts of BCAAs than younger, as it is considered that the elderly are at higher risk for nutritional deficiencies, something that may impact BCAA metabolism. [59] Regarding the potential effect of BCAAs in renal and liver insufficiency, it is unclear whether BCAAs supplementation promotes the synthesis of albumin and apo B100 in cirrhosis and nonalcoholic steatohepatitis. [60,61] In total, although several clinical conditions could benefit from BCAAs consumption, there are no reliable markers to evaluate or quantify their requirements such that the duration and dose of their supplementation be capable of being monitored.

3.8. Administration Protocol

Based on scientific knowledge to date, exact proportions, consumption protocols, and absolute recommendations cannot be given as there are no common recruitment guidelines from all International Sports Nutrition Organizations. The official position of ISSN (2007) proposed a daily consumption of ~25 gr BCAA in a ratio of 2:1:1 (leucine, isoleucine, and valine), if possible before any intense exercise (endurance or resistance). [24] Also, the recommended daily dose for leucine alone should be 45 mg/kg/day for sedentary people, and even higher to much higher for physically active people. However, while more research is needed because BCAAs are also found in nature (i.e. through the consumption of animal protein) in a ratio of 2:1:1 (leucine, isoleucine, and valine), one can consider swallowing ≥45 mg/kg/day of leucine, along with approximately ≥22.5 mg/kg/day of both isoleucine and valine over a 24-hour time frame to optimize overall training adjustments. [24] This will ensure the 2:1:1 ratio that is often found in animal protein. If adequate daily protein consumption requirements are met (≥1.6 gr/kg/day), it is considered that no apparent benefits from additional BCAA intake exist, as muscle hypertrophy and strength-related performance require a full complement of essential amino acids. [63] Consuming high-protein meals that contain all essential amino acids will maximally stimulate muscle protein synthesis. [49] Also, it should not be overlooked that complete food

proteins, as well as most quality protein powders, contain about 25% BCAAs. Any deficiency in BCAA intake from food can be easily remedied by consuming whey protein during the time frame covering the exercise period. Finally, an effort should be made for the athlete to obtain all recommended amounts of BCAAs from complete food protein sources.

3.9. Possible Side Effects

Excessive protein intake (>3 gr/kg/d) may cause several adverse effects, such as kidney damage, elevated blood lipoprotein levels (associated with atherosclerosis), and dehydration. The latter can occur as a result of increased urinary nitrogen release, resulting in increased urinary volume and dehydration. Therefore, athletes on a high-protein diet should increase their fluid intake to prevent dehydration. Recommended protein intake for athletes (1.2 to 1.9 gr/kg/d and up to ~2 gr/kg/d) does not appear to be harmful. Acute intake of BCAA supplements at doses of about 10-30 gr/d does not appear to have a detrimental effect. According to Sports Dieticians Australia, up to date, no studies have been shown that BCAAs supplementation harms athletes' health.

4. Nitrate

Nitrate (NO_3^-) is a bioactive compound (inorganic polyatomic anion) that exists naturally in the environment. It occurs in both air and drinking water, as well as in certain foods, and is produced endogenously by a family of nitric oxide synthetase (NOS) through the oxidation of the amino acid l-arginine. [12] Nitrates are found mainly in the diet (>80%) as minerals of vegetables. [65] The predominant sources of nitrates are beets, celery, lettuce, radishes, and spinach, and are more likely to be found in green vegetables grown in greenhouses or hydroponically. [65] NO_3^-, through its conversion to nitrite (NO_2^-), is also a common food preservative found in bacon, hot dogs, and pre-made meat dishes. [65] Nitrite consumption is mainly found exogenously through the consumption of cereals, vegetables, and cooked (smoked) meat products. NO_3^- and NO_2^- have been used for centuries to preserve the flavor and color of processed and seasoned meats and have exhibited antimicrobial and antifungal properties. [12] The last decade, dietary NO_3^- and its metabolites were recognized in sports nutrition science for their advantageous effects on athletic performance and health. [66,67] Deteriorated data claiming unfavorable effects of NO_3^- supplementation has provoked scientific discussion as to whether it is beneficial or unsafe to human health. [68,69]

4.1. Mechanisms of Action

Until relatively recently, it was believed that the ubiquitous normal signaling molecule, nitric oxide (NO), was produced only by the oxidation of L-arginine in a reaction catalyzed by NOS resulting in the endogenous production of nitrates (NO_3^-) and NO_2^-. [66] However, it is now known that nitrates and nitrites can be converted back to NO and other oxides of bioactive nitrogen in vivo, and there is growing scientific interest in the potential of this pathway "nitrate-nitrite-NO" in physiology, diet, and medicine. [65] It has been suggested that this alternative route may complement the L-arginine-NOS-NO pathway, allowing NO production in conditions of low oxygen availability in which NOS (oxygen-dependent) NOS activity may be reduced. [4] In addition to its production through the NOS system, the body reserves of nitrates and nitrites can also be increased exogenously through diet, and mainly through the consumption of green leafy vegetables such as lettuce, spinach, arugula, celery, cardamom, and beets, which typically contain more than 250 mg (4 mmol) of nitrate per 100 g of fresh weight. The ingested inorganic nitrates circulate in the plasma, and a portion of them (~25%) is received by the salivary glands and concentrated in saliva. [66] Following the ingestion of nitrates, the concentration of nitrates in plasma reaches its peak after 1-2 hours while the concentration of nitrites reaches its peak in plasma after 2-3 hours. After this period both gradually decrease reaching back to their original values after about 24 hours. [15,18] A variety of enzymes and proteins catalyze the reduction of a nitrite electron to NO in the blood and other tissues. This process is facilitated in conditions of low oxygen availability (ischemia and hypoxia) and low pH, which allows NO to be produced where it is most needed. [70] Interestingly, these conditions (low pressure of oxygen and pH) may be present in skeletal muscle during exercise. [19]

4.1.1. Nitrates and Mechanisms of Action to Enhance Performance

The mechanisms by which nitrates act to improve the response of muscle metabolic efficiency are a rather complex issue. It has been suggested that enhanced NO levels (due to nitrate intake) may reduce the ATP/PCr cost associated with skeletal muscle strength production. [70] Research, using the spectroscopy method for in vivo analysis of quadriceps muscle exercise, found a significant reduction in muscle accumulation of [Pi] and [ADP] after ingestion of nitrates in the form of beetroot juice. [72] Their findings showed a 21% reduction in [Pi] for maximal muscle contractions during low-intensity exercise and a reduction in PCR stock depletion after beetroot intake. [72] Another proposed possible mechanism by which nitrates directly alter mitochondrial efficiency is via attenuating the P:O ratio. Reducing the gross oxygen cost of exercise has been hypothesized to be achieved through reduced proton leakage around the mitochondrial membrane. [73]

4.2. Nitrates and Athletic Performance

In the market of sports, nutritional aids are available in several nitrates forms which contain nitrates, either alone or in combination with other substances in the form of powder or liquid oral solution. The scientific findings support their consumption, as it is considered that enhancing the bioavailability of NO through activation of the nitrate-nitrite-NO pathway can affect muscle function and performance in exercise. [17,18,19,20] In theory, NO can regulate skeletal muscle function through its role in regulating blood flow, muscle contractility, calcium and glucose homeostasis, and mitochondrial respiration and

biogenesis. [15] In vivo, inhibition of NOS, which would reduce endogenous NO production, increases oxygen consumption in dogs and rats.[18] In humans, the effect of NOS blockade is more controversial, but there is evidence that NO is involved in regulating blood flow and VO2. [70] This increases the likelihood that increased NO bioavailability could positively affect athletic performance. [70] Indeed, several studies have reported that plasma nitrite concentration is positively associated with exercise ability in humans. [71] These findings raise the possibility that increasing plasma nitrites through nitrate supplements may have ergogenic effects. [71] A review of 29 studies that examined the potential effects of nitrate supplementation on exercise tolerance or athletic performance showed a significant effect of its supplementation on exercise tolerance as compared to the placebo group, while, presented no significant effect on exercise performance (Effect Size=-0.05; 95%CI:-0.28 to 0.17; p=0.64) than placebo. [74] In similar, another review study indicated that nitrate supplementation enhances plasma nitrite concentration, decreases resting blood pressure and the oxygen cost of sub-maximal exercise, while it can enhance athletic performance and exercise tolerance. [71] A most recent meta-analysis by Tan et al. (2022) speculated that 40% of sprint-type studies showed improved power output, sprint time, and total work in running or cycling, while 40% of the resistance-type studies incorporated improvements to strength, velocity, and power of resistance exercise. [18] Other meta-analyses revealed an ergogenic effect of NO_3^- supplementation in recreationally active, young men; although, the effect size was objectively small (d=0.174), proposing that its supplementation has deteriorated utility as an ergogenic supplement in subjects with high aerobic fitness. [19] It is widely accepted that beetroot is an excellent source of dietary nitrate (mean value of 1446 mg/kg of fresh weight). [75] A meta-analysis by Wong et al. (2021) examining beetroot supplementation on athletic performance revealed no significant improvement to mean or peak power output during high-intensity interval training or sprint interval training. [20] Another recent review (2019) speculated that the effect of beetroot supplementation on exercise performance is contradictory, as the time to exhaustion seems to improve but its effect on time-trial athletic performance needs more clarification. [16] Moreover, the authors concluded that individuals with low aerobic fitness levels might gain higher benefits in athletic performance. [16] Inline, a study examined the administration of NO_3^- to national level cyclists and their response to high-intensity exercise showed that NO3- can be particularly ineffective and perhaps even detrimental to the high-intensity exercise of those who compete at a very high level. [76] In contrast, other studies have now reported that NO_3^- (given mainly through beetroot juice) [Beet Root Juice, BRJ] can improve end times, exercise time to exhaustion, and increase maximal strength and exercise rate. [77-82] Many of these studies have also been conducted by elite cycling athletes and highlight the use of NO_3^- supplementation to improve athletic performance in various sports (e.g. cycling, running, rowing, static apnea testing). [77-82] Overall, chronic (3-15 days) and acute (2-3 h) nitrate administration either as NO_3^- rich beetroot juice (5.1-18.1 mmol NO_3^- per dose) or as $NaNO_3^-$ (0.1 mmol/kg is associated with improvements in maximal exercise in walking, running, rowing and cycling and to improve tolerance to more intense exercise rates in almost all age groups of both sexes in trained and untrained populations. Most studies use doses of 70 ml BRJ. [77-82] Also, it is of great concern the findings of a recent review study speculated that although women are underrepresented in dietary NO_3^- supplementation investigation, there are sex differences in response to NO_3^- supplementation. [83]

Regarding the NO_3^- supplementation dose issues, a study by Wylie et al., (2016) showed that no improvement in the physiological response to exercise was achieved with NO_3^- doses below ~5 mmol. In addition, no additional benefit appears to be achieved from doses greater than ~8-9 mmol. These findings suggest that doses in the 5-9 mmol range tend to be more beneficial for those seeking improvement in exercise. Moreover, acute administration (2.5-3 hours) of BRJ (~5.0-6.2 mmol NO_3^-, single dose) has also shown ergogenic benefits (e.g. lower VO_2, increased exercise tolerance, increased oxygenated blood levels) at 4.0 and 16.1 km of sub-maximum intensity cycling exercise, while similar benefits have been observed even at simulated altitudes of 2,500 meters. Most studies used doses of 500 mL (~2 cups) of BRJ with varying concentrations of NO_3^- (5-18 mmol NO_3^-/day, and mainly 5-8 mmol NO_3^-/day), with performance improvements observed mainly for time-to-exhaustion protocols with constant power exercise (e.g. cycling, running, walking, etc.) at sub-maximum and high intensities. [77-82]

The variability of the potential ergogenic effects of NO_3^-administration on athletic performance has several explanations. Some authors have suggested that failure to elicit an ergogenic effect may be directly related to the variability of individual responses between participants (e.g. different aerobic fitness levels). [19] These findings may suggest that athletes, ranging from amateurs to elites, males or females, may require higher doses of NO_3^- or may be more resistant to NO_3^- supplementation. It should also be noted the dose and the time that NO_3^- has been administrated which probably leads to smaller increases in plasma NO_2 levels, while a larger dose may prove more beneficial. [19] Furthermore, while some of these studies showed possible indications of NO_3^- ergogenic aid, many of these parameters failed to reach statistically significant levels. [19]

4.3. Nitrates and Health

Many chronic human diseases include a dysfunctional endothelium and the inability to adequately produce and maintain NO homeostasis. In this regard, a new trend seems to be emerging in nutrition science, suggesting that along with reduced calorie and fat intake, diets rich in foods that stimulate NO bioavailability (e.g., NO_3^- and NO_2^- enriched foods) are good choices that can offer a variety of health benefits. [85] Indeed, along with daily physical activity, dietary intake should be considered as the first goal in preventing disease. In the United States, the NO_3^- dietary intake is estimated to be about 31-185 mg per day, with about 85% coming exclusively from vegetables. [15] This intake can be greatly enhanced to

reach over 1200 mg (~20 mmol), including high amounts of NO_3^-, as part of the dietary approach to stopping hypertension. [15] It is noteworthy that this intake is about 5 times higher than the accepted daily intake of the World Health Organization, which is 3.7 mg NO_3^- per kilogram of body weight per day. [15] Most interestingly, however, almost identical (and sometimes even greater) reductions in hypertension have been observed with moderate doses of dietary NO_3^- although much more research is needed to finalize this effect. [15] Indeed, NO_3^- is probably the main ingredient in vegetable-rich diets and probably one of the many responsible for its beneficial effects on blood pressure. Moreover, it proposed that BRJ has anti-inflammatory and anti-cancer, and has a lower risk for harmful cardiovascular outcomes such as myocardial infarction, stroke, and the formation of gastric ulcers. [85,86,87] In addition, given that under conditions of senescence or illness, the production of NO was reduced, it is considered that exogenous NO supplementation may have a therapeutic effect for individuals undergoing senescence or illness. [88] Finally, a review study revealed that among other beneficial effects dietary NO_3^- supplementation could have an advantageous effect on exercise training in patients with heart failure, peripheral artery disease, and chronic pulmonary obstructive disease. [16] Until recently, however, NO_3^- remained largely neglected in terms of its beneficial properties. But it is a key nutrient inherent in vegetable and fruit-rich diets, such as the Mediterranean and traditional Japanese diets, which have long been linked to protection against cardiovascular disease and type-2 diabetes.

4.4. Possible Side Effects

Nitrates and nitrates are naturally present in vegetables and are also added to processed meats to "enliven" the color and delay spoilage. The possibility of nitrosamines forming in foods through their enrichment with nitrates has stimulated the debate about the safety of nitrite intake. There is only limited data on the effects of nitrate supplements on athletes due to the production of nitrosamines. [15] It should be noted that any possible harmful effect of nitrogenation (assuming the presence of secondary amines in saliva or stomach) is very effectively inhibited by the antioxidants that coexist with nitrates in vegetables. [89] However, although nitrates themselves are non-toxic due to their limited and slow conversion to nitrite, there is the potential for toxicity with accidental or uncontrolled use of nitrite. [90] Scientific opinions so far agree that nitrate supplements in combination with plant products such as beetroot juice are unlikely to be harmful. [89] Therefore, athletes wishing to explore the potential ergogenic properties of nitrate supplements are advised to use natural herbal products for this purpose.

4.5. Recommended Doses

Based on current research data, it is proposed: [15-20,71]

• Nitrate supplementation at a dose of 8-12 mmol/day for 6 to 15 days appears to be likely to have a positive effect on athletic performance, especially in aerobic exercise. Daily intake of 5-9 mmol of nitrates can be achieved through diet without the use of supplements.

• The effects of acute NO_3^-supplementation on athletic performance are not as consistent.

• The beneficial effects of NO_3^- intake appear to be related to those sports in which aerobic exercise lasts from 5 to 30 minutes.

• Whether longer-term NO_3^- supplementation may support or enhance the physiological adaptations to exercise training is at present unidentified.

• There are no scientific findings to suggest a dose-dependent relationship between NO_3^-dosing and athletic performance.

5. Conclusions

BCAAs and nitrates have become increasingly popular mainly for their potential effect on individuals' health and secondary as ergogenic aids. BCAAs supplementation seems to be recommended for all athletes who exercise vigorously daily. Based on sufficient scientific data, it enhances their recovery after causing exercise-induced muscle damage. Also, taking BCAAs supplements reduces the feeling of delayed muscle pain, which follows because of muscle damage. Limited scientific research findings suggest a potentially beneficial effect of BCAAs on reducing central fatigue. Although there is considerable encouraging evidence, the positive effect of BCAAs supplementation on athletic performance indicators does not appear to be fully established. Scientific data proposed that several clinical conditions could benefit from BCAAs consumption; however, there are no reliable markers to evaluate or quantify their requirements such that the duration and dose of their supplementation be capable of being monitored.

To date, no serious adverse effects on the health of athletes have been reported due to BCAAs supplementation. There are no exact consumption protocols and absolute recommendations, mainly because BCAAs are also obtained through the consumption of animal protein.

Dietary nitrates can lower blood pressure, reduce the cost of exercise oxygen, and, at least sometimes, enhance exercise capacity. Also, very little is known about optimal nitrate intake, which can optimize these positive outcomes while minimizing any health risks. For example, while it is known that supplementation of approximately 5-9 mmol of nitrate/day for 1-15 days may have beneficial effects on normal exercise responses, the exact dose-response relationship has not yet been established. It should also be noted that 5-9 mmol of nitrates can be easily consumed in the normal diet and there is currently no evidence to adequately document that taking additional nitrates produces greater benefits. Regarding the effect of nitrates on exercise performance in healthy volunteers, the literature seems to agree that taking supplements for 2-6 days (or up to 15 days) can increase athletic performance during high-intensity exercise. The effects of acute supplementation on athletic performance are less consistent, as some studies show a positive result, while others do not show any effect. The effectiveness of acute

nitrate supplementation is likely to depend on several factors such as age, sex, health, diet, and level of fitness/training experience (including the proportions of muscle fiber types, and baseline nitrate levels in plasma) of the subjects, the intensity, duration and nature of the training, and also whether the exercise is done in a norm or hypoxia. Whether long-term nitrate intake may support or increase physiological adjustments to exercise are currently unknown. The duration of continuous maximum exercise for which nitrates appear to be ergogenic is between 5-30 min. There is limited evidence that nitrates are beneficial in prolonged exercise performance (40 min), at least when administered short term.

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Conflict of Interest

No conflict of interest

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